VASCULAR EFFECTS OF ENDOTOXINS*

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IN Table I are listed some of the more striking biological phenomena produced by injections of bacterial endotoxins. It has become increasingly clear over the past two decades that many of these phenomena are mediated by vascular reactions of one sort or another. The pyrogenic effect of small doses of endotoxin and the shock produced by larger doses are both associated with profound vasomotor changes.[1] The local reaction to injections of endotoxin is characterized by striking alterations in the local blood vessels.[2] The dermal Shwartzman reaction, in which a large area of skin exhibits a peculiar sort of hemorrhagic necrosis, has been shown to depend on a sequence of changes in local blood supply,[2] while the so-called generalized Shwartzman reaction likewise is due to severe blood vessel damage and occlusion.[3] The hemorrhage and necrosis observed in experimental tumors after intravenous injection of endotoxin appears to be secondary to vascular damage[4] as do several other effects of endotoxin. The purpose of this paper will be to examine what is now known of the vascular effects of endotoxin, and to indicate to what extent these effects may constitute common pathogenetic mechanisms in the various phenomena produced by endotoxin.

In Table II are listed some of the well-established effects that need to be considered. All of these effects can be demonstrated within minutes or hours after the injection of endotoxin, and nearly all have been demonstrated in several species of animals, including man, so that they may be regarded as characteristic and potentially significant components of the overall host response. It would be misleading to suggest that all of the important vascular effects of endotoxin have been identified, but this table indicates some that may be involved in the mechanism of the phenomena listed in Table I, and these will be considered in turn.

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TABLE I—SOME PHENOMENA PRODUCED BY ENDOTOXIN

1. Fever
2. Shock
3. Dermal Reaction
4. Shwartzman Phenomena:
   (a) Local Hemorrhagic Necrosis
   (b) Bilateral Renal Cortical Necrosis
5. Tumor Hemorrhage
6. Abortion
7. "Toxic Ocular Reaction"
8. Increased Resistance to Shock ("Tolerance")
9. Increased Antibody Response ("Adjuvant Effect")
10. Increased Resistance to Infection (Nonspecific)

TABLE II—SOME "VASCULAR" EFFECTS OF ENDOTOXIN

1. Vasomotion
2. Increased Vascular Permeability and Stickiness
3. Hyperreactivity to Epinephrine
4. Hyporeactivity to Epinephrine
5. Leucopenia and Thrombocytopenia
6. Intravascular Agglutination of Leucocytes and Platelets
7. Thombosis
8. "Heparin-Precipitable" Fibrinogen and "Fibrinoid"
9. Altered Histidine Decarboxylase Activity
10. Fibrinolysis

The vasomotor changes that have been described include a profound peripheral vasoconstriction that begins abruptly several minutes after an intravenous injection of endotoxin and lasts from several minutes to an hour or more, depending on the dose of endotoxin given.[1] The vessels then usually undergo dilation and the muscular arteries of the rabbit's ear, for example, may exhibit segmental vasomotion for some time thereafter. These changes are said not to occur in the denervated rabbit's ear, and can be largely prevented by the administration of adrenergic blocking agents.[1] In a thorough series of investigations, Zweifach and his collaborators[5-7] have shown that these vasomotor effects of endotoxin are apparently due to a biphasic alteration in sensitivity of vascular smooth muscle to epinephrine, small doses of endotoxin rendering vessels extremely sensitive to epinephrine *in vivo* and in the isolated perfused rabbit's ear as well, while with larger doses of endotoxin the epinephrine sensitivity first increases far above normal, then declines to a point where the vessels cannot be stimulated to contract even with enormous amounts of epinephrine. There are many reasons for believing that this altered reactivity to epinephrine is a basic and meaningful feature of the vascular response to endotoxins.

Some years ago it was shown that local intradermal injections of endotoxin lead to the development of delayed inflammatory reactions, resembling the "tuberculin" type of allergic reaction.[2] When epinephrine is infiltrated into such lesions, massive hemorrhage and necrosis ensue, while normal skin suffers no irreversible damage when injected with the same amount of epinephrine. Thomas subsequently showed[8] that the intradermal injection of a mixture of endotoxin and epinephrine causes severe local hemorrhagic necrosis, and that still another combination, endotoxin injected intravenously followed by epinephrine injected intradermally, produced similar local tissue damage. These phenomena all reflect an altered reactivity of blood vessels to epinephrine, but the mechanism of the tissue damage in each case is not yet entirely clear.

The production of generalized increased vascular permeability and endothelial stickiness is apparently another effect of endotoxin. Experiments with transparent chambers in rabbit's ears have shown that intravenous injections of endotoxin are followed by the adherence of leucocytes to capillary and vein walls. This is clearly a complicated phenomenon, both the leucocytes, as isolated from the peripheral blood,[9] and vascular endothelium, as tested separately,[10] are sticky. This generalized stickiness of vascular endothelium and of leucocytes and platelets is a really dramatic feature of the systemic reaction to endotoxins. Clumps of agglutinated leucocytes and platelets can be seen in the peripheral blood stream, and the profound leucopenia and thrombocytopenia which develop are due to the trapping out of these sticky masses in peripheral capillary beds, paricularly in the lung.[2]

The cause of the stickiness has been investigated by biophysicists, immunologists and others, and there is no general agreement as to its cause or nature. One of the most venerable and still popular concepts is that somehow the clotting mechanism is involved, the stickiness being due to a surface coating of fibrin or altered fibrinogen. That endotoxin can initiate and accelerate the clotting of whole blood *in vitro* has been clearly demonstrated[11] and alterations of the clotting mechanism *in vivo* have also been found. Perhaps the most striking such alteration is the demonstration by Thomas and collaborators[3] that after intravenous injection of endotoxin much of the circulating fibrinogen is so altered that it precipitates in the cold in the presence of heparin. This precipitation is reversible, and the altered fibrinogen can also be precipitated by admixture with other acidic polysaccharides. Many animals find some

way of laying down this material as an amorphous "fibrinoid" deposit in glomerular capillaries. This happens more frequently after the second of two intravenous injections of endotoxin, whereupon nearly every glomerular capillary is completely occluded by the material. This obstruction to blood flow naturally causes infarction of the renal cortex, or bilateral renal cortical necrosis. This phenomenon in animals is usually called the "generalized Shwartzman phenomenon", but it is probable that the same mechanism of tissue damage is at work in human cases of bilateral cortical necrosis.

The more classical dermal Shwartzman phenomenon involves quite another mechanism of tissue damage. Here, too, the vascular effects of endotoxin are primarily involved, but in a different way. This phenomenon is produced by the intradermal injection of endotoxin in a rabbit, followed by a period of waiting. During this time an inflammatory response slowly develops, reaching its peak at about 24 hours. A second injection of endotoxin, given intravenously this time, causes a dramatic series of events. Petechiae and purpuric spots develop in the involved skin area, which rapidly undergoes hemorrhagic necrosis.[12]

Much work has been done to provide an understanding of this deceptively simple phenomenon, as it seems to many to provide a useful experimental model for the study of tissue damage in certain human infectious diseases. It has been shown that thrombosis is involved, since the phenomenon can be inhibited by anticoagulant therapy.[13] It has been shown that vasomotor reactions are involved, since the phenomenon can be reproduced by injecting vasoconstrictor agents into the prepared skin site[2] and can be blocked or modified by adrenergic blocking agents. It has been shown that polymorphonuclear leucocytes are involved, since treatment with x-ray or radiomimetic drugs prevents the phenomenon under circumstances clearly implicating these cells.[14] The mechanism seems to be no more complicated, however, than a simple combination of some of the vascular effects already discussed. The intradermal injection leads to an inflammatory reaction in which the walls of small veins become sticky. The intravenous injection produces vasoconstriction, sluggish blood flow and plugging of these vessels by masses of leucocytes and platelets. The complete cessation of blood flow results in death of the tissue, which has demanding metabolic properties. When the animal recovers from the peripheral circulatory collapse and pumps blood into this dead tissue, massive extra-

vasation through necrotic vein walls occurs and the full-blown picture of hemorrhagic necrosis is seen.[2]

The phenomenon of induced hemorrhage and necrosis in tumors, brought about by endotoxin, seems to have a similar basis. Here the tumor, with a glycolytic metabolism closely resembling that of inflamed tissue, is equally dependent for survival on an adequate blood supply. When an intravenous injection of endotoxin is given, vasoconstriction occurs, endothelium of tumor vessels becomes sticky and traps leucocytes and platelets until the vessel is actually occluded by a "white thrombus". Death of the tumor, with subsequent hemorrhage into the necrotic tissue, proceeds as before. It is possible that some of the reactions to Coley's toxin a generation ago were due to this mechanism.

These are some of the more drastic phenomena produced by endotoxin, and they quite obviously involve vascular reactions of a complicated nature—vascular endothelium and vascular smooth muscle are very intimately involved in most of these reactions, while intravascular stickiness and aggregation of leucocytes and platelets on the one hand and fibrinogen and perhaps other clotting factors on the other are conspicuously involved in one or another of them. I have deliberately avoided the subject of shock because, while endotoxin in modest amounts will indeed produce irreversible shock and death in many species, there is at the moment a lively controversy over the subject of whether endotoxins from intestinal bacteria are involved in hemorrhagic or traumatic shock. An adequate treatment of this problem is outside the scope of this presentation, although to be sure, the production of shock is among the most dramatic of the vascular effects of endotoxin.[1, 5-7]

There is little to be gained at the moment by discussing the mechanism by which intravenous injections of endotoxin produce striking changes in the eye of the rabbit[15] or abortion by the pregnant mouse.[16] While there is not doubt that vascular effects are involved in these phenomena as well, it would, perhaps, be more to the point to raise questions concerning the mechanism by which endotoxin induces vascular effects in the first place.

Does endotoxin have a direct effect on vascular smooth muscle, on endothelium or on leucocytes or platelets? The answer seems to be, in each case, that it does not. Each of the vascular effects that we noted in Table II is apparently itself a secondary effect, resulting from some

TABLE III—SOME DIRECT IN VITRO EFFECTS OF ENDOTOXIN

1. Initiation of Coagulation
2. Stimulation of Leucocyte Respiration and Glycolysis
3. Sensitization to Epinephrine

basic biological activity of which we now know almost nothing. So that, although we can explain the phenomenon of renal cortical necrosis, for example, in terms of vascular effects (vasoconstriction, formation of "heparin-precipitable" fibrinogen), we are at a loss in turn to explain how endotoxin produces vasoconstriction or "heparin-precipitable" fibrinogen. Some few effects of endotoxin have been demonstrated *in vitro* (Table III) but in each case again appear to be indirect effects. The effect on coagulation is apparently mediated by natural antibodies, since it can be mimicked by other antigen-antibody complexes and cannot be produced in the absence of antiendotoxin antibodies.[11] The stimulation of leucocyte metabolism in shed whole blood[17] is similarly antibody-dependent. In fact, it has been suggested that perhaps the biological activity of endotoxin may be entirely immunological in nature; that is, that endotoxins represent a class of bacterial antigens so ubiquitous that mammals are "naturally" hypersensitive to them.[18] While there is a good deal to be said for this point of view, there are some telling arguments against it. For example, the chick embryo is easily killed[19] by microgram quantities of endotoxin (death occurs as a result of extensive and severe vascular damage, incidentally) and the newborn rabbit is fully susceptible to the lethal and some of the other effects of endotoxin.[18] Endotoxin can hardly be acting in these cases as an antigen to which the animal is hypersensitive, as we now conceive of hypersensitivity. Work on the biochemistry and toxicology and immunology of endotoxins, however, is almost certain to provide us in the foreseeable future with the insight that we need to understand these vascular effects of endotoxins.

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